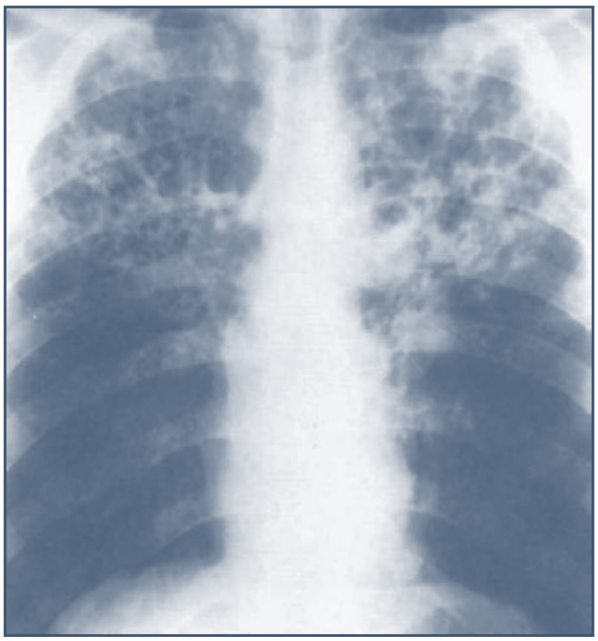
TUBERCULOSIS

NIAID plays a lead role in the NIH tuberculosis (TB) research program. In response to ongoing concern about increasing worldwide case rates and the development of multidrug-resistant strains of *Mycobacterium tuberculosis (M.tb),* the pathogen that causes TB, NIAID has increased its TB research portfolio steadily over the past decade. The World Health Organization (WHO) estimates that there are approximately 8 million new TB cases annually, with 2 million deaths. This toll makes TB the leading cause of death from a single infectious pathogen worldwide, killing more people than AIDS and malaria combined. Approximately one-third of the world's population is infected with *M.tb*, and 1 in 10 of these individuals likely will develop active TB disease in their lifetimes. If current trends continue, an estimated 1 billion people will be newly infected by the year 2020; approximately 200 million people will develop active TB, and 35 million will die.[65]

NIAID supports a broad TB research program, primarily through its extramural Division of Microbiology and Infectious Diseases (DMID), with particular emphasis on the following areas:

- Basic biology and pathogenesis of *M.tb*, host-pathogen interaction, and host response to TB in animal models and humans;
- Research into the various stages of TB, including persistent, asymptomatic infection with *M.tb* (latency), reactivation, and progression to TB;
- Development and testing of vaccines, chemotherapeutics, and diagnostics;
- Development of improved tools for epidemiologic studies; and
- Mycobacterial genomics and postgenomic analyses.



Lung showing lesions caused by infection with *Mycobacterium tuberculosis.*

Recent funding increases have allowed the Institute to support a number of initiatives and to markedly expand the community of TB researchers. Higher levels of funding enabled NIAID to establish the Tuberculosis Research Unit (TBRU) at Case Western Reserve University in 1994 (*www.tbresearchunit.org*). TBRU continues to make progress in developing surrogate markers of disease and human protective immunity, and in conducting clinical trials of potential new TB therapeutic, preventive, and diagnostic strategies. Activities of the TBRU are coordinated with other major organizations involved in TB research, including the Centers for Disease Control and Prevention, U.S. Agency for International Development, U.S. Food and Drug Administration (FDA), WHO, Global Alliance for TB Drug Development, and International Union Against Tuberculosis and Lung Disease, and with interested industrial partners.

NIAID's extramural TB research program currently supports more than 200 grants for basic, applied, and clinical TB research. Among the projects supported by NIAID is an award to The Institute for Genomic Research in Rockville, Maryland, to support sequencing and annotation

of *Mycobacterium smegmatis* (strain MC2 155), an important model system used in TB research. *M. smegmatis* microarrays are produced under this grant and were distributed through the Pathogen Functional Genomics Resource Center in FY 2004. For a current list of available microarrays, which includes *M. smegmatis,* see *http://www.niaid.nih.gov/dmid/genomes/pfgrc/guidelines.htm.* For access to genome data, see *www.tigr.org/tdb/mdb/mdbinprogress.html.*

The development of improved TB vaccines, which are crucial to the long-term control of TB worldwide, is a high priority. In December 2003, NIAID, together with the FDA Center for Biologics Evaluation and Research, sponsored a workshop to outline U.S. regulatory requirements for the development and human testing of new TB vaccines (*http://www.niaid.nih.gov/dmid/meetings/tbvacc.htm*). The NIAID Blueprint for TB Vaccine Development, presented at the 1998 International Symposium for Tuberculosis Vaccine Development and Evaluation, outlines the specific steps needed to develop improved TB vaccines (*http://www.niaid.nih.gov/publications/blueprint*). A Department of Health and Human Services-wide task force, which includes representation from NIAID, oversees implementation of the blueprint report. Clinical trials of two new TB vaccines that were developed with NIAID support began in 2004. One is a recombinant version of the bacillus Calmette-Guerin vaccine, developed by investigators at UCLA; the other is an adjuvant-peptide fusion vaccine developed by Corixa Corporation.

Through the Tuberculosis Research Materials and Vaccine Testing contract with Colorado State University, NIAID provides TB research reagents and preclinical vaccine testing services to qualified investigators throughout the world. By the end of FY 2004, more than 150 new TB vaccine candidates had been tested under this contract, one of which has recently entered human clinical trials with several others progressing through various stages of preclinical development.

Contracts issued by DMID and the Division of Acquired Immunodeficiency Syndrome (DAIDS) are used to support and promote a range of TB studies from basic through translational to applied research. Under the contracts, NIAID (1) offers *M.tb*-derived research reagents and animal model screening services for candidate TB vaccines (*http://www.cvmbs.colostate.edu/microbiology/tb/top.htm*); (2) offers candidate compound identification and acquisition services, and in vitro and animal model screening services to evaluate drug candidates (*http://www.taacf.org*); (3) provides funding for the development of improved TB vaccines using already existing technology platforms; (4) supports TBRU to conduct multidisciplinary laboratory and clinical studies to answer critical questions about human TB; to provide knowledge, tools, and technologies to improve human clinical trials in TB; and to provide the capability to conduct clinical studies for the evaluation of new or improved vaccines, therapeutics, and diagnostics (*http://www.cwru.edu/affil/tbru/index.htm*); and (5) assists with technology transfer for potential commercialization of new drug discoveries for TB.

Under the NIAID contracts mentioned above, the Southern Research Institute maintains the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) to acquire drug candidates for screening against virulent *M.tb*, maintains a computerized chemical database of candidate structures, coordinates and distributes compounds for evaluation *in vitro* and in animal models, and reports data to compound suppliers. TAACF has contacted more than 3,500 chemists throughout the world seeking candidate anti-TB compounds. TAACF has received more than 70,000 compounds from academic and private-sector investigators, principally in the United States and Europe, with growing involvement of scientists from Africa, Asia, Australia, South America, and other regions.[66]

NIAID supports a high-throughput, robotics screening contract that continues to provide

screening services to discover new antimicrobials. The facility supported under this contract provides the capability of testing large chemical libraries of compounds for activity against specific biochemical drug targets and against growing microorganisms. In addition to supporting *in vitro* evaluation of compounds from chemical repositories, DAIDS also recently awarded two research grants to stimulate preclinical research for novel therapeutic strategies against TB in the context of HIV/AIDS.

A new component of the NIAID TB drug-development support services was awarded in 2004 under the Pharmacokinetics and Pharmacodynamics Animal Model contract. This contract supports a central facility to identify and evaluate novel compounds for their basic pharmacology and efficacy characteristics, provide critical support for investigator-initiated drug discovery, stimulate private-sector sponsorship of new drugs, perform comparison and confirmatory studies from different sponsors, and provide information for the selection of antimicrobial drug candidates for designing clinical studies.

NIAID also participates in a newly formed public-private partnership—the Global Alliance for Tuberculosis Drug Development (*http://www.tballiance.org*)—together with WHO, the Rockefeller Foundation, and other international organizations dedicated to encouraging new therapeutic advances in the absence of industrial sponsorship. In addition, increased funding through Small Business Innovation Research grants has promoted development and evaluation of new tools for treating and preventing TB.

DAIDS is supporting clinical trials of new treatment and prevention strategies for tuberculosis in the setting of HIV/AIDS. These investigations are being conducted in countries with a high burden of disease associated with both TB and HIV. The interactions of these two infections are associated with high mortality, particularly in African nations. In 2004, DAIDS awarded one grant under the International Studies of AIDS-Associated Co-infections program, which is designed to develop effective and sustainable clinical management strategies to improve care and foster integration of research on HIV and co-infection pathogens including tuberculosis. The Comprehensive International Program of Research on AIDS supports research studies addressing important public health research questions in high-burden countries.

The Division of Allergy, Immunology, and Transplantation (DAIT) supports a number of individual research projects concerned with basic mechanisms of immunity to *M.tb*. DAIT's research goals and objectives on *M.tb* are as follows:

- Understand how the immune system recognizes and responds to bacteria such as *M.tb*, hidden within host cells, and support research on antigen presentation and stress molecule induction as they relate to activation of cell-mediated immunity to intracellular pathogens;
- Promote vaccine-relevant research to identify dominant mycobacterial antigens and novel adjuvants that induce protective cellular immune responses;
- Promote research on the development of immunologic reagents for early diagnosis and monitoring of disease; and
- Support research on the identification of immune system genes that activate in response to mycobacterial infection, especially genes that encode soluble proteins that might be relevant to the development of TB vaccines or therapies.

Research topics include T-lymphocyte recognition of mycobacterial lipid antigens, the role of various cell populations in combating *M.tb* infection, and the function of biological oxidants in protective immune processes.

DAIT supports several projects that assist research on TB as well as other infectious diseases such as hepatitis C, malaria, and HIV. Under the Application of Data on Human Leukocyte Antigen (HLA) to the Improvement of Vaccines program, DAIT supports the HLA Ligand/Motif Online Database, a Web-based, searchable database of human major histocompatibility complex (MHC) molecules and peptide ligands. The database specifies amino acid sequences of peptides derived from viral, bacterial, parasitic, and human proteins in association with human class I or class II MHC molecules. This resource enables users to search for specific human MHC/peptide combinations or to determine ligand amino acid motifs that will facilitate their research. Support is provided under a NIAID contract to the University of Oklahoma.

The NIAID Tetramer Facility produces peptide-MHC reagents for T cell detection. Reagents this facility supplies are relevant to many vaccine-related topics, including intracellular bacterial, viral, and parasite infections; autoimmune diseases; and basic immunobiology. More information about this facility can be found at *http://www.niaid.nih.gov/reposit/tetramer/index.html*. The National Cancer Institute also provides funding for the Tetramer Facility.

The Division of Intramural Research (DIR) has a substantial intramural program that integrates genomics and combinatorial chemistry to speed development of new antibiotics for the control of TB. After contributing to the determination of the genomic sequence of *M.tb*, DIR investigators are now focusing on unraveling the functions of its various genes. This knowledge is critical to new drug and vaccine development and to understanding the molecular mechanisms involved in disease pathogenesis and the emergence of drug resistance. For example, despite the apparent lack of significant genetic differences among *M.tb* strains, there is mounting evidence that considerable variation exists in *M.tb* molecules that are important in disease pathogenesis. These differences may allow some *M.tb* organisms to modify the host cellular immune response and thereby contribute to the observed diversity of tuberculosis disease. NIAID intramural scientists identified and described the functional relevance of a molecule—a phenolic glycolipid (PGL)—produced by a subset of *M.tb* organisms that shows "hyperlethality" in murine disease models. Disruption of PGL synthesis resulted in loss of hyperlethality and correlated with an increase in the release of substances that help the immune system fight *M.tb* infection. These findings demonstrate that the spectrum of TB disease observed in humans is likely to reflect not only variable host factors, but also the variable expression of bacterial factors, including PGL.

DIR scientists also are working on a number of different approaches to improve current TB drug therapies and to develop new drugs. This work will be facilitated by their development of the first comprehensive tool for the elucidation of the molecular mechanism of action of anti-tubercular drugs. Using DNA microarrays, the scientists characterized the *M.tb* genes that were transcribed or "turned on" in response to all known anti-TB drugs—thus revealing a molecular signature for each drug type that could be used to find new compounds with a similar signature. They also found that the transcriptional profile generated by a crude marine natural product predicted the mode of action of the pure active component. This tool will allow researchers to gain an immediate appreciation of the mechanism of an unknown agent and will greatly facilitate the drug discovery process.

DIR scientists also are continuing a project with colleagues from GlaxoSmithKline and St. Jude's Children's Hospital to develop an improved anti-tubercular drug based on thiolactomycin, a compound isolated from a soil bacterium. DIR collaborators at St. Jude's used X-ray crystallography to determine the structure of thiolactomycin bound to its enzyme target. Using this structure as a guide, scientists are now synthesizing and testing derivatives of thiolactomycin that might be more active against

TB than thiolactomycin itself. This partnership is a model for the development of drugs against diseases that lack the financial impact necessary to attract independent attention from the global pharmaceutical industry. DIR scientists also have partnerships with colleagues from South Korea, Cambodia, and Nigeria to collaborate in studies of multidrug resistance and TB-HIV co-infection. In South Korea, an institutional review board (IRB) has been formed, a TB natural history clinical research protocol is in the final stages of IRB approval, and clinical research training of staff has been completed.

NIAID support for TB research has led to significant advances in our understanding of the basic biology, microbiology, and immunology of TB, which will result in the development of new diagnostic tools, vaccine candidates, and therapeutic strategies to prevent and ultimately cure this devastating disease.